

COST Action FA1406 Phycomorph

Advancing knowledge on seaweed growth and development

Third Working Group Meeting

Ghent University

Phycology Research Group

2 – 4 October 2017

Abstract Book



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Abstract Book of the Third Working Group Meeting
COST Action FA1406 “PHYCOMORPH” Programme

Editor: Xiaojie Liu & Jonas Blomme

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Scientific programme of the meeting

Monday (02.10.2017) Avenue Louise 149, 1050 Brussels, Belgium		
13:00	Registration WG meeting	
14:00	Welcome & Introduction to Phycomorph B. Charrier & R. Robaina, Chair & Co-chair COST Action FA1406	Opening
14:15	1. The <i>Ulva</i> genome: insights in the life of a bloom forming alga Olivier de Clerck, Univ Ghent, Belgium	WG4. Advancing technical tools Chairs: Christos Katsaros & Rita Araujo
14:40	2. Use of monoclonal antibodies to study the cell walls of brown algae Thomas Torode, Univ Cambridge, U-K	
15:05	3. The <i>Chara</i> genome: implications for plant terrestrialization Noe Fernandez Pozo, Univ Marburg, Germany	
15:30	Coffee Break	
16:10	4. NA interference: a potent tool for studying gene function of genetically non-transformable algae as shown in <i>Euglena gracilis</i> Adeel Nasir, Univ Erlangen, Germany	WG4. Advancing technical tools Chairs: Christos Katsaros & Rita Araujo
16:35	5. Towards genetic transformation of the brown alga <i>Ectocarpus siliculosus</i> by PEG transformation Aude Le Bail, Univ Erlangen, Germany	
17:00	6. Development of CRISPR-Cas9 in the multicellular green algae <i>Ulva mutabilis</i> Jonas Blomme, Univ Ghent, Belgium	
17:25	7. Uncovering the genetic basis of bacteria-induced morphogenesis in <i>Ulva mutabilis</i> Michiel Kwantes, Univ Jena, Germany	
17:50	End of Day 1	

Tuesday (03.10.2017) Avenue Louise 149, 1050 Brussels, Belgium

09:00	8. Sidestepping reproduction: using sterility to unlock the growth and metabolic potential of <i>Ulva rigida</i> . Gary Caldwell, Univ Newcastle, U-K	WG1. Fertility Chairs: Fabio Rindi & Juliet Coates
09:40	9. Volatiles in red seaweeds: A world truly incredible to discover Pilar Garcia-Jimenez, Univ Las Palmas, GC, Spain	
10:05	10. Genetic and cellular characterization of parthenogenesis in the brown alga <i>Ectocarpus</i> Laure Mignerot, Station Biologique Roscoff, France	
10:30	Coffee Break	
11:10	11. Lifecycle controls of kelp Alexander Ebbing, NIOZ, The Netherlands	WG1. Fertility Chairs: Fabio Rindi & Juliet Coates
11:35	12. Ouroboros and samsara trigger the gametophyte-to-sporophyte transition in the brown alga <i>Ectocarpus sp.</i> Simon Bourdareau, Station Biologique Roscoff, France	
12:00	13. Developmental and ecological response of <i>Fucus vesiculosus</i> to environmental contamination in the northwest Atlantic Jessie Lauze, Univ Massachusetts Dartmouth, USA	
12:25	LUNCH(Sandwiches)	
14:00	14. Genetic and hormonal control of vascular cell proliferation in <i>Arabidopsis thaliana</i> Bert De Rybel, VIB, Belgium	WG2.Reproduction and initiation of the new generation Chairs: Olivier De Clerck & Aschwin Engelen
14:40	15. Title: Two-phased cell polarisation in the brown alga <i>Dictyota</i> Kenny Bogaert, Univ Ghent, Belgium	
15:05	16. Investigating the role of the cell wall in <i>Dictyota dichotoma</i> embryo patterning Marina Linardic, Univ Cambridge, U-K	
15:30	Coffee Break	
16:10	17. What makes an <i>Ectocarpus</i> filament grow? Enlightenment from biophysical modeling and simulation Bénédicte Charrer, Station Biologique Roscoff, France	WG2. Reproduction and initiation of the new generation Chairs: Olivier De Clerck & Aschwin Engelen
16:35	18. Seaweeds – ‘second skin’ and the resulting Volatilome Mahasweta Saha, Univ Essex, U-K	
17:00	19. Improving the Cultivation Techniques of <i>Porphyra dioica</i> in an Integrated Multi-Trophic Aquaculture System in Portugal Jessica Knoop, Univ Swansea, U-K	
17:25	20. <i>Ulva</i> protein kinases: what we know (and don't know) so far Juliet Coates, Univ Birmingham, U-K	
17:40	End of Day 2	
19:00	Dinner at “The Quincaillerie” http://www.quincaillerie.be/fr/	

Wednesday (04.10.2017) Avenue Louise 149, 1050 Brussels, Belgium

09:00	21. Physical constraints as a driver of cellular proliferation patterns in the bloom-forming green alga <i>Ulva spp.</i> Kevin Yun, Univ Durham, U-K	WG3. Towards Adult Growth Chairs: Thomas Wichard & Helena Abreu
09:25	I22. Fluorescent staining of actin cytoskeleton in vegetative cells of the filamentous brown algae <i>Ectocarpus siliculosus</i> Hervé Rabillé, Station Biologique Roscoff, France	
09:50	23. Effects of co-occurring hydrodynamic and nutrient stresses on morphology and biomechanics of <i>Saccharina latissima</i> Guorong Zhu, NIOZ, Netherlands	
10:15	Coffee Break	
10:55	24. Developmental and ecological differentiation in cryptic seaweed species Christophe Viera, Ghent Univ, Belgium	WG3. Towards Adult Growth Chairs: Thomas Wichard & Helena Abreu
11:20	25. Nutrients transport in the <i>Ulva</i> species macroalgae Meiron Zollman, Tel Aviv Univ, Israel	
11:45	26. A dual Periphyton-Ulva biofilter for mariculture effluent Ben Shahr, Nat. Center for Mariculture Eilat, Israel	
12:10	LUNCH(Sandwiches)	
14:00	27. Macroalgae cultivation in Norway: Research on industrial development and environmental impact with relevance for guidelines Aleksander Handa, SINTEF, Norway	Workshop/Preparati on Of Seaweed Aquaculture guidelines Coordinators: Susan Holdt & Michèle Barbier
14:15	28. Norwegian Integrated Multi-Trophic Aquaculture (IMTA) from pilotscale to a profitable industry Céline Rebours, Moreforks, Norway	
14:30	29 Macroalgal standardization in EU Susan Holdt, DTU, Denmark	
14:45	30. Breeding risks in aquaculture Bertrand Jacquemin, CEVA, France	
15:00	31. Access and use of marine resources for scientific research, legislation and ethics Michèle Barbier, EU Ethics expert, France	
15:15	DISCUSSION and identification of the main issues;Preparation of the next workshop (including coffee break)	
17:00	B. Charrier & R. Robaina	Closure
17:05	End of Day 3	

1. The *Ulva* genome: insights in the life of a bloom forming alga

Olivier De Clerck

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Ulva, probably the best known representative of the green seaweeds (Ulvophyceae), is renowned for its spectacular green tides under high nutrient conditions. Despite the negative connotation as a nuisance species, *Ulva* is also traditionally cultivated for human consumption ('aonori') and increasingly used for removal of nutrients in integrated aquaculture systems. *Ulva* is also progressively becoming a model organism to study morphogenesis in the green seaweeds. The *Ulva*-thallus is relatively simple, with uninucleate cells and a limited number of cell types. The morphogenesis however is dependent on the presence of bacteria, without whom cell division and cell wall formation is repressed. A transformation system for *Ulva* has been developed, making it an attractive complementary system for functional biology in the green lineage. Here I will report on the 74MB haploid genome of *Ulva mutabilis* and link the information of the genome to its biology. I will focus on aspects of multicellularity, plant hormone signaling and the capacity of *Ulva* to attain extremely high grow rates.

2. Use of monoclonal antibodies to study the cell walls of brown algae

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The cell walls of the brown algae are a heterogeneous composite, composed primarily of polysaccharides. Two of these polysaccharides are unique to the cell walls of the brown algae (sulphated fucans and alginate). Monoclonal antibodies have extensively been used to study the cell walls of land plants due to their capacity to recognise distinct glycan structures. In order to dissect the composition, abundance and architecture of the cell walls of the brown algae, a range of monoclonal antibodies (BAMs) have been raised against sulphated fucans (BAM1-5) and alginate (BAM-11). These new tools have been used in a variety of in muro and in vitro assays to dissect the immense variety of brown algal cell walls.

3. The *Chara* genome: implications for plant terrestrialization

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Land plants evolved from charophyte algae. Among charophytes, the Zygnematales, Coleochaetales and Charales are most closely related to land plants, with the Charales having the most complex body plans. Here, we present the draft genome of *Chara braunii* (Charales). Comparison of the genome to those of land plants and other algae identified evolutionary novelties for land plant terrestrialization and land plant heritage genes. We find that *C. braunii* employs unique xylan synthases for cell wall biosynthesis, that the phragmoplast mechanism is similar but not functionally identical to that of land plants, and that many phytohormones and regulators identified in land plants are present. *C. braunii* plastids are controlled *via* plant-like retrograde signaling, and transcriptional regulation is more elaborate than in other algae. The striking morphological complexity of this organism may result from expanded gene families, with three cases of particular note: a large set of genes effecting tolerance to reactive oxygen species (ROS), LysM receptor-like kinases, and transcription factor families. Transcriptomic analysis of sexual reproduction reveals intricate control by transcription factors, activity of the ROS gene network, and the ancestral use of plant-like storage and stress protection proteins in the dormant stage of the zygote.

4. NA interference: a potent tool for studying gene function of genetically non-transformable algae as shown in *Euglena gracilis*

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Algae are unique biomass producers in the food chain. However, the stable genetic transformation of microalgae and macroalgae is a great challenge. Because of this restraint, several important studies such as localization of protein and gene silencing could not be performed in several algal model organisms. Even though various emerging technologies claim to obtain gene silencing in a range of organisms, they are still in their early developmental stage. We suggest the well-established and robust technology of RNA interference as a tool to study gene function in algal cells with an example of *Euglena gracilis*. *E. gracilis* is a single cell eukaryotic photosynthetic flagellate belonging to the group of the Disciscritates. Several published results indicate the successful and reproducible results of gene silencing in *E. gracilis* performed by RNAi. Here, we will present the data regarding the determination of genes involved in the gravity sensing mechanism of *E. gracilis* using RNAi.

5. Towards genetic transformation of the brown alga *Ectocarpus siliculosus* by PEG transformation

Aude Le Bail¹, Bénédicte Charrier², Benedikt Kost¹

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The brown alga *Ectocarpus siliculosus* and the moss *Physcomitrella patens* share remarkable developmental similarities. Both are composed of single cell filaments elongating by tip growth that differentiate into two cell types. These similarities might suggest that common cellular mechanisms are employed to develop such structures. The *E. siliculosus* mutant *etoile*, composed mainly of round cells (Le Bail et al., 2011), has a mutation in a Rho - GAP gene (regulatory protein of the Rho GTPase signaling pathway, unpublished), while the quadruple mutant of the four Rho - GTPases of *P. patens* shows a similar phenotype with a complete loss of polar growth (unpublished). In an evo/devo context, it is tempting to test whether *P. patens* proteins involved in the Rho - GTPase pathway are functional in *E. siliculosus* and vice versa. Although the genetic transformation is perfectly established in *P. patens*, *E. siliculosus* has not yet been successfully transformed, preventing the two directional evo/devo approaches. In this context, a short term scientific mission has been carried out to develop the PEG mediated genetic transformation in *E. siliculosus*. The moss transformation protocol has been tested and adapted to the survival of the brown algal gametes and the methods developed in the green algal *Ulva mutabilis* (Oertel and al, 2015) have also been performed. A range of plasmids has been used, carrying *E. siliculosus* promoters or commonly used promoters driving either a mutated actin gene leading to the resistance to Latunculin B or the reporter gene GFP.

6. Development of CRISPR-Cas9 in the multicellular green algae *Ulva mutabilis*

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Ulva mutabilis (sea lettuce) is an emerging model organism for green macroalgae. Not only is *Ulva* an important species in coastal benthic ecosystems around the world, it is cultivated as food and feed, for bioremediation, in integrated aquaculture systems but also as a putative biofuel (Wichard et al., 2015). Recently, the small genome of *Ulva mutabilis* (~101Mbp, De Clerck et al. in prep.) has been sequenced and annotated (10,481 coding genes). Interestingly, *Ulva* unmated gametes are ideal for genetic manipulation and reproducible standardized experiments as they develop parthenogenetically into clonal, haploid gametophytes. Moreover, *Ulva* has a short generation time (3-5 weeks) and has been stably transformed using vector plasmids (Oertel et al., 2015). Now, the greatest challenge for molecular genetics research on *Ulva* is expanding the genetic toolkits and the efficient generation of mutant lines. We aim to develop CRISPR-Cas9 and generate stable *Ulva* mutant lines for genes involved in inorganic carbon acquisition. Given the universal and flexible nature of our cloning system, different vectors can be created to characterise the generated mutants in detail. In this project, growth and development of the mutant lines will be studied. Developing genetic research tools allows to complement traditional physiological research and will further help establishing *Ulva* as a model system for green seaweeds.

References:

Oertel, W., Wichard, T., and Weissgerber, A., 2015. Transformation of *Ulva mutabilis* (Chlorophyta) by vector plasmids integrating into the genome. *J. Phycol* 51, 963–979.

Wichard, T., Charrier, B., Mineur, F., Bothwell, J.H., Clerck, O.D., and Coates, J.C., 2015. The green seaweed *Ulva*: a model system to study morphogenesis. *Front Plant Sci* 6.

7. Uncovering the genetic basis of bacteria-induced morphogenesis in *Ulva mutabilis*

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The green marine macroalga *Ulva mutabilis* (Chlorophyta) requires the presence of two specific bacteria, or the morphogenic compounds they produce, in order to achieve normal development. Although the identification of the molecules that mediate this mutualism steadily progresses, the genetic circuitry that underlies it still needs to be elucidated. To investigate which *Ulva* genes are involved in the regulation of bacteria-dependent morphogenesis we performed a forward genetic screen. After genetic transformation, based on the integration of a phleomycin resistance marker in the genome, *Ulva* individuals that survived selection and potentially showed aberrant mutant phenotypes were recovered. Next, we tested different molecular methods to identify genomic insertion sites and determined that adapter-ligation PCR is the most efficient way to retrieve these regions. Using this approach, mutants were identified that carry insertions in intergenic - repetitive - sequences, but, interestingly, also in genes encoding an LRR receptor-like protein and a WD40-repeat-containing protein. Subsequent *in silico* analysis showed that homology of these genes with other (Chlorophyta) genes was mostly limited to the parts encoding canonical protein domains. Therefore, although this comparison did not shed light on the molecular function of the proteins, it does suggest that the identified genes are unique to *Ulva*, which is in line with its distinctive dependence on bacteria for morphogenesis. Currently, we are further studying to which extent gene function in these mutants is disrupted and how it affects the *Ulva* phenotype.

8. Sidestepping reproduction: using sterility to unlock the growth and metabolic potential of *Ulva rigida*.

Gary S. Caldwell

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The green seaweed, *Ulva*, is highly valued in terms of animal feed, food and biofuel, as well in the delivery of crucial remediation services including wastewater treatment and CO₂ removal. Accordingly, *Ulva* cultivation has gained significant research interest worldwide. Notwithstanding these research efforts, *Ulva* cultivation is still in its infancy and knowledge to underpin such developments remains limited. A common challenge in *Ulva* cultivation is the fluctuating productivity with time due to vegetative fragmentation and/or periodic reproductive events. Here, the culture performance of a sterile strain of *U. rigida* was obtained by mutating a wild strain with ultraviolet radiation. This new strain grew five times faster over an 18-day cultivation and absorbed nitrate and phosphate 40.0% and 30.9% more rapidly compared to the wild strain respectively. The chemical composition of the sterile strain showed a lipid content of more than double that of the wild strain, while the protein content was 26.3% lower than the wild strain. These findings will be discussed in relation to the continued development of *Ulva* cultivation within the scope of a changing global ocean.

9. Volatiles in red seaweeds: A world truly incredible to discover

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Different volatile organic compounds in marine algae can act as priming volatile growth regulators that affect reproduction in seaweeds. Taking into consideration the myriad of volatile compounds released, we have focused on those ones affected by physiological conditions related to sporogenesis, namely ethylene and methyl jasmonate. Their effects in red seaweeds, as an increment in the tetrasporangial branches in *Pterocladia capillacea* and in the cystocarps number in *Grateloupia imbricata*, and the presence of mixed phases in *Gelidium sp.*, are showed. To continue, we will make a genetic dissection of how far seaweeds can discern between different volatiles and the molecular mechanisms underlying carposporogenesis in *G. imbricata*. We are aware that more information and research are needed but amazing perspectives have been opened up.

10. Genetic and cellular characterization of parthenogenesis in the brown alga *Ectocarpus*

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While transitions between sexual and asexual reproduction are believed to have important evolutionary and ecological consequences, the molecular, genetic, and cytological foundations of such transitions remain largely uncharacterized. One type of asexual reproduction is parthenogenesis, i.e, the development of an adult organism directly from gametes in absence of fertilisation. Although many eukaryotes may reproduce by parthenogenesis, we know very little about its genetic basis, and the evolutionary causes and consequences of transitions to asexuality are poorly understood. The brown algae are a group of largely understudied multicellular eukaryotes, that show an extraordinary diversity of types of life cycles, sexual systems, modes of reproduction, and they provide excellent models to look at the origins, evolution and mechanisms underlying parthenogenesis. In this study, we exploited the wide array of genomic and cell biology tools available for the model brown alga *Ectocarpus* to identify and characterize the loci involved in parthenogenesis and to shed light into the causes and consequences of parthenogenesis at the organism level.

11. Lifecycle controls of kelp

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The lifecycles of Phaeophyceae, and in particular the controls to their lifecycles, are largely understudied research topics. With a European seaweed industry that is steadily growing, it is increasingly essential to understand and control their entire lifecycle. Sporogenesis and the sexual reproduction between gametophytes are major lifecycle transitions that are therefore of particular interest.

Here we will discuss future studies within the scope of this PhD project and focus on the growth, sexual reproduction of gametophytes and the influence of light quality on sexual reproduction. The access to 2 different species (*S.latissima* and *A.esculenta*) originating from 7 different places along a latitudinal gradient will make in-depth comparative studies possible.

This talk will furthermore focus on previous studies conducted on this specific subject. Emphasis is placed on their results, their differences, missing information, and the subsequent gaps in knowledge. A new strategy will hereby be developed on how to tackle missing information on the lifecycle controls of the Phaeophyceae.

12. Ouroboros and samsara trigger the gametophyte-to-sporophyte transition in the brown alga *Ectocarpus sp.*

Simon Bourdareau¹, Delphine Scornet¹, Laurent Pérès¹, Josselin Guéno¹, Leïla Tirichine Delacour², Bérangère Lombard³, Damarys Loew³, José M. Franco-Zorilla⁴, Susana M. Coelho¹, J. Mark Cock¹

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Most brown algae (a stramenopile lineage) exhibit haploid-diploid life-cycles that involve alternation between two multicellular organisms; the diploid sporophyte and the haploid gametophyte. In *Ectocarpus sp.* each generation develops from individual cells called zoids. Meiospores released after meiosis by mature sporophytes develop as gametophytes. Males and females gametes produce by matures gametophytes can fuse to form diploid zygotes. To function coherently, the pathways that control the transitions between the sporophyte and gametophyte must be initiated at the appropriate stage of the life-cycle. Genetic analysis of mutants of *Ectocarpus* has identified two major life-cycle regulators called *OUROBOROS* (or *ORO*) and *SAMSARA* (or *SAM*). Both are predicted to encode TALE homeodomain-containing transcription factors (HD-TFs). Loss-of-function mutants exhibit homeotic switches where a fully functional gametophyte replaces the sporophyte generation. These two TFs are therefore necessary to trigger the gametophyte-to-sporophyte transition through fertilization. Interestingly, TALE HD-TFs are involved in the haploid-to-diploid transition in other distant eukaryotic supergroups suggesting that *ORO* and *SAM* may be representatives of an ancient regulatory system of broad importance across eukaryotic tree. The objective of this project is to elucidate molecular processes associated with the morphological gametophyte-to-sporophyte transition in *Ectocarpus*.

13. Developmental and ecological response of *Fucus vesiculosus* to environmental contamination in the northwest Atlantic

Jessie Lauze, Whitney Hable

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Brown algae provide important habitats to intertidal communities. *Fucus vesiculosus* is prevalent in rocky intertidal zones of the northeastern United States, where algal beds provide feeding and nursery habitats for commercially important fish and invertebrate species. Due to commercial and residential expansion along coastal waterways, organisms in these habitats are often subject to pollutant exposure. Within New Bedford Harbor, on the southeast coast of Massachusetts, high levels of polychlorinated biphenyls and heavy metals have contaminated coastal ecosystems for decades. Although declared a priority cleanup site by the United States Environmental Protection Agency, these ecosystems remain laden with dangerously high levels of contaminants. Very few studies have explored how this contamination might affect primary productivity, despite the fact that *Fucus vesiculosus* has been historically abundant in the New Bedford Harbor area.

Our studies examined the reproductive capacity of mature individuals and the ecological condition of populations with varying levels of exposure to the most common environmental contaminants. We also investigated the ability of zygotes produced in these varying exposures to meet developmental landmarks. Poor biological condition (delayed development, impaired or arrested ability to release gametes, and decreased biomass and density) was strongly correlated with increased contaminant load. Findings suggest recovery of populations from heavily contaminated areas is unlikely, but also provide insight into the ability of these fucoid algae to survive in these habitats.

14. Genetic and hormonal control of vascular cell proliferation in *Arabidopsis thaliana*

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The plant vascular system develops from a handful of provascular initial cells in the early embryo into a whole range of different cell types in the mature plant. In order to account for such proliferation and to generate this kind of diversity, vascular tissue development relies on a large number of highly oriented cell divisions. Control of these divisions occurs in part through the TARGET OF MONOPTEROS 5/ LONESOME HIGHWAY (TMO5/LHW) dimers of bHLH transcription factors and their homologs. The cytokinin (CK) biosynthetic gene *LONELY GUY4* (*LOG4*) and its close homolog *LOG3* were identified as direct targets of the TMO5/LHW dimer complex, indicating that CK biosynthesis plays a crucial role in this developmental process. Here, I will highlight our current progress in understanding how cell division orientation is controlled during vascular development.

15. Title: Two-phased cell polarisation in the brown alga *Dictyota*

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In most complex eukaryotes development commences with the establishment of cell polarity that determines the first axis of the future body plan. The underlying mechanisms of polarity establishment are still emerging. Using a combination of microscopy, pharmacological experiments and transcriptomic analyses we show that cell polarity in the brown alga *Dictyota* is established in a two-phased process with the first phase narrowing down the possible polarisation vectors to a set of two. Like in land plants, the zygote of *Dictyota* establishes the apical-basal axis during the first cell division. Upon egg activation, the zygote undergoes an F-actin/myosin dependent, 90s lasting elongation along a maternally determined axis that is reflected in the cytoplasmic distribution of plastids. Which of the two poles of the resulting prolate spheroidal zygote will acquire the basal cell fate, is determined environmentally as assessed by the direction of unilateral light. Addition of auxins induces multiple rhizoids under conditions of low pH and changing light direction, suggesting a role for auxins in apical-basal patterning. The second phase of cell polarisation is accompanied and dependent on zygotic transcription instead of uniquely on maternal factors. In that embryogenesis in brown algal plant systems is more similar to higher plants rather than animal system. Cell polarisation as observed in *Dictyota* whereby determination of direction and sense of the polarisation vector are mechanistically and temporally decoupled, in two distinct processes is unique.

16. Investigating the role of the cell wall in *Dictyota dichotoma* embryo patterning

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Brown algae are a group of multicellular photosynthetic organisms which, despite their different evolutionary paths, share a number of developmental features with plants; e.g. cell walls. The cell wall plays a key role in regulating algal and plant developmental processes by maintaining cell shape, withstanding turgor pressure and modulating its structure to allow growth. My project investigates the role of cell walls in *Fucus serratus* embryos by studying their biochemical and mechanical properties, growth dynamics and division patterns during early development. The STSM mission at the University of Ghent has introduced me to an additional brown algal species – *Dictyota dichotoma*. I have learnt how to cultivate *Dictyota* vegetatively in Provasoli enriched seawater (PES) and how to perform gamete release and fertilisation. In addition, I have learnt how to control growth of unwanted species such as diatoms and bacteria. Adapting these techniques to my two *Fucus serratus* systems (embryos and regenerated branches) has enabled me to improve their culturing. Interestingly, preliminary results have shown that adding nutrients (PES) did not affect the growth of embryos, but significantly increased the growth of regenerated branches. By applying the knowledge obtained in Ghent to my project, I gained valuable skills in brown algal culturing. Knowing how to culture *Dictyota* and using it as an additional model for cell wall research in the future, could bring an even deeper understanding about the role of cell walls in brown algae.

17. What makes an *Ectocarpus* filament grow? Enlightenment from biophysical modeling and simulation

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Here, we used a biophysical simulation approach⁽⁵⁾ to show that, in contrast, the brown alga *Ectocarpus*⁽⁶⁾ has evolved an alternative tip-growth mechanism involving a finely tuned gradient of cell-wall thickness along the cell that can compensate for the *Ectocarpus* is a filamentous brown alga in which development is initiated by tip growth, a cellular process encountered in pollen tubes, root hairs, fungal hyphae, metazoan neurons and oomycete filaments⁽¹⁾. This is the most widely distributed unidirectional growth process on the planet. It ensures spatial colonization, nutrient predation, fertilization and symbiosis with growth speeds of up to 800 $\mu\text{m}\cdot\text{h}^{-1}$ ⁽²⁾.

Although turgor-driven growth is intuitively conceivable, a closer examination of the physical processes at work in tip growth raises a paradox: growth takes place where biophysical forces are low, due to the increase in curvature in the dome⁽³⁾. To compensate this drawback, all tip-growing cells studied so far increase cell-wall extensibility via polarized excretion of loosening enzymes at the tip⁽⁴⁾.

We used a viscoplastic biophysical model⁽⁵⁾ to numerically simulate tip growth in this alga. The model integrates several biological parameters, such as the internal cell pressure, the cell shape and the isotropic organisation of the cell wall. Based on the measurements carried out *in situ* on living algae, numerical simulations showed major differences in the strategies developed by this alga compared to the other tip-growing organisms.

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18. Seaweeds – ‘second skin’ and the resulting Volatilome

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Volatile organic compounds (VOCs) are important ‘infochemicals’ (information-conveying chemicals) that play a crucial role in structuring life on our planet and fulfil diverse functions in natural and artificial systems. However, environmental volatile metabolomics (volatilomics) is poorly explored for the assessment of structure and function in marine systems. Seaweeds and their associated epibacteria (their ‘second skin’) are known to contribute significantly to the global budget of volatiles that are important for climate functioning. We developed an analytical pipeline based on gas chromatography (GC-MS) to characterize biogenic volatile organic compounds (BVOCs) from seaweeds. Freshly collected phaeophyte, chlorophyte and rhodophyte seaweeds (*Fucus vesiculosus*, *Ulva lactuca*, *Ceramium* sp.) showed taxon-specific volatile metabolomes. The sulfur gas dimethyl sulfide (DMS) was a significant feature in the chlorophyte whereas the phaeophyte and rhodophyte showed lower production. Principal Component Analysis (PCA) demonstrated clear separation of algal genera based on their volatilome signatures. A similar study with artificially-induced seaweed beach wracks from *Fucus vesiculosus*, *Ulva compressa*, *Ceramium* sp. showed clear separation of volatilome signatures generated by different species composition of beach wracks.

Due to technical difficulties of separating a seaweed host from its epibacteria, the majority of studies on seaweed-bacteria chemical interactions have addressed the volatile chemicals released by the seaweed-epibacterial holobiont as one entity. Thus, we lack fundamental understanding of the contributions made by each partner and how the interactions change when these two partners are united. Thus, in my STSM project, I analysed the volatilomes of axenic and non-axenic *Ulva* and their epibacteria using SPME and Purge-and-Trap methodologies. PCA analysis revealed differences among the axenic, non-axenic and their epibacteria volatilome.

19. Improving the Cultivation Techniques of *Porphyra dioica* in an Integrated Multi-Trophic Aquaculture System in Portugal

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Red algae of the genus *Porphyra/Pyropia* are commonly farmed in Asia, while cultivation is still in its infancy in Europe. Focusing on establishing the knowledge base for *Porphyra* cultivation in South Wales and granted with a Short Term Scientific Mission, a joined project was initiated to evaluate alternatives to the use of autoclaved seawater and nutrients to cultivate the filamentous conchocelis sporophyte of *P. dioica*. We also assessed growth and productivity performance of *P. dioica* gametophytes grown at different stocking densities in an Integrated Multi-Trophic Aquaculture system. *P. dioica* conchocelis were cultivated at three different nutrient regimes (no addition of nutrients, addition of half-strength von Stosch's (VSE), addition of full-strength VSE). Growth and development was followed for three weeks. Furthermore, *P. dioica* gametophytes were cultivated at three different stocking densities (0.5, 1.0 and 2.0g·l⁻¹). Growth rates and productivity were followed over two weeks. The addition of nutrients enhanced conchocelis growth and development. Gametophyte growth rate was highest (19.5±1% day⁻¹) at lowest stocking densities (0.5g·l⁻¹) and decreased with increasing stocking density. Productivity increased inversely to growth rates with highest productivity (0.66±0.12g DW l⁻¹week⁻¹) at a stocking density of 2g·l⁻¹. Therefore, total productivity increased with stocking density despite lower relative growth rates. The addition of media, even at reduced concentrations, is crucial for a successful maturation of the filamentous conchocelis stage. Low productivity of gametophytes in the low stocking densities compared to high productivity but low growth rates in high stocking densities suggest best stocking densities at 1.0g·l⁻¹ under the given cultivation conditions.

20. *Ulva* protein kinases: what we know (and don't know) so far

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Protein kinases are enzymes that phosphorylate target proteins on serine, threonine, tyrosine or histidine residues. They are key players in cell signalling and multicellular development/cell interactions in eukaryotes, including land plants and animals. In unicellular organisms, kinases have important roles in environmental/stress signal transduction and in flagellar movement.

We have identified all the protein kinases (the “kinome”) in the multicellular green seaweed *Ulva*. We are currently comparing characteristics of *Ulva* protein kinase families to unicellular algal kinomes and land plant kinomes. We see increases in cyclin-dependent kinase family members in multicellular algae.

21. Physical constraints as a driver of cellular proliferation patterns in the bloom-forming green alga *Ulva spp.*

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Ulva spp. is a genus of multicellular green algae that is present in much of the coastal benthic zones worldwide with extremely plastic morphology, and even within species thalli undergo different morphogenesis (Mshigeni and Kajumulo, 1979; Hoeksema and van, 1983; Ansell et al., 1998; Xu et al., 2012; Zhang et al., 2012). *Ulva spp.* only has three cell types (blade, rhizoid and stem) arranged into two opposing one-cell thick sheets, without differentiation into tissues and as such, there are no active mechanisms of cell signalling or overarching cellular architecture coordination within the plant itself (Wichard et al., 2015). *Ulva spp.* is a symbiotic organism, and its morphogenesis requires cross-kingdom signalling between itself and bacteria, and though the exact mechanisms are at this stage unknown, *Ulva* will grow as undifferentiated lumps, that are atypical of their morphology, in the absence of associated bacteria (Burke et al., 2009; Joint et al., 2007, 2002).

The lack of overarching cellular architecture coordination within the plant itself gives rise to an imminent question: is the morphogenesis entirely coordinated by symbiotic bacteria, or is the morphogenesis driven partly or wholly by physical mechanisms, rather than biological ones? The latter is the subject of investigation in our experiments. Cell distribution patterns were investigated with confocal microscopy using the dye Direct Yellow 96. Using this information and mathematical modelling in order to elucidate the possible underlying mechanisms, we wish to ask two main questions: 1. How does the natural tessellation pattern compare to an optimal Centroidal Voronoi Tessellation? and 2. How do the natural tessellation patterns differ in different morphotypes?

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22. Fluorescent staining of actin cytoskeleton in vegetative cells of the filamentous brown algae *Ectocarpus siliculosus*

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Tip-growth is a highly polarized form of cell morphogenesis, in which cell wall deposition and extension are restricted to one pole of the cell. In my thesis, I study the mechanisms of tip-growth in *Ectocarpus siliculosus*, a basic model species for brown algae, in order to compare it with tip-growth mechanisms in the more intensively studied land plants and fungi. Using Latrunculin B treatments, we observed that actin filaments (AFs) were essential for tip-growth in *E. siliculosus*. In order to decipher the exact mechanisms by which AFs control tip-growth, I need to determinate their detailed structure in apical cells. AFs have never been observed in *E. siliculosus* so far, so a new staining method for this cytoskeleton component was required. Moreover, actin staining is tricky, especially in plant cells, because of AFs rapid degradation. As part of a Phycomorph STSM, I traveled to the laboratory of Pr. Christos Katsaros, of the University of Athens, in which an actin fluorescent staining protocol was developed for some other brown algal species. During that STSM, this protocol was successfully adapted to *E. siliculosus*. The next step consists in transferring this protocol to the home lab (Roscoff), in order to help me reaching my PhD objectives. In parallel, the protocol was written to be disseminated in the book “*Protocols for Macroalgae Research*” (in press, ISBN-13: 978-1-4987-9642-2).

23. Effects of co-occurring hydrodynamic and nutrient stresses on morphology and biomechanics of *Saccharina latissima*

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Hydrodynamic and nutrient stresses are two major contributors to the growth, distribution and even the survival of seaweeds. The interactive effects of waves, nutrient levels as well as nutrient fluctuations on the morphology and the biomechanics of 3 size classes of *Saccharina latissima* were investigated in an 8-week laboratory experiment. Results showed that wave exposure was the primary factor increasing almost all the morphological (e.g. blade and stipe length) and biomechanical (e.g. toughness, stiffness) parameters. Among the 3 size classes, the more sensitive common morphology and blade shape ratio were observed in smaller and larger individuals. Along the central lamina of the thallus, the new tissue produced prominently changed blade shape and force-extension curve, especially when exposed to waves. Our experiments with *Saccharina latissima* exposed to different hydrodynamic forces and nutrient availabilities revealed that waves are the primary factor influencing the morphology and biomechanics. Nutrient availability on the contrary had only a slight influence. Overall, *Saccharina latissima* appeared to be highly plastic in response to the environmental stresses applied.

Relevant Working Group: Towards adult growth

24. Developmental and ecological differentiation in cryptic seaweed species

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The use of molecular tools is revealing an unexpected diversity in many groups including seaweeds. Cryptic species, morphologically indistinguishable and ecologically alike, exhibit little niche differentiation, making it difficult to explain their co - occurrence. To better understand to what extent cryptic lineages coexist, we investigate interspecific developmental and ecological differentiation within a group of brown seaweed, the genus *Lobophora* (Dictyotales, Phaeophyceae). Several species of *Lobophora* that co - occur extensively in the Pacific were selected to study their reproduction and development. Several aspects are investigated, including the study of the life cycle, the effect of abiotic factors (temperature, light and salinity) on vegetative growth, fertility induction and spore development. Here, we present some preliminary results of this ongoing research work.

25. Nutrients transport in the *Ulva* species macroalgae

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Rapidly growing energy consumption has led in recent years to a realization of the urgent need to develop alternative renewable energy sources. One of the important but least explored alternative energy resources lies within the offshore production of biomass. Unlike the more investigated microalgae, macroalgae cultivation can be done offshore and therefore brings real news to the biofuel - land agriculture conflict. Nutrients are of major importance for the growth rate and chemical composition of the algae, and more so in the oligotrophic Mediterranean Sea. In these nutrients' scarcity conditions, nutrients should be used wisely and efficiently, emphasizing the need for enhanced knowledge regarding uptake and assimilation mechanisms and kinetics. My research relates to this need and strives to: 1. characterize and model *Ulva*'s nutrient uptake and assimilation mechanisms and kinetics, and 2. optimize sugar production and growth rate through control of nitrogen flux and water flow. Within the frame of this work, one of the major technical challenges, to which the STSM allowed a possible solution, is nutrient measurement methods suitable for low, seawater, concentrations. During my 5 days mission in NIOZ, Yerseke, I learnt how to use a segmented flow autoanalyzer (SFA) and prepared a user manual. Acquiring this tool enhances my basket of methods necessary to deal with one of my largest measurement challenges, thus promoting my research.

26. A dual Periphyton-Ulva biofilter for mariculture effluent

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The environmental impact of mariculture is one of the major obstacles for the expansion this agro-industry. Additional constraints are the economic problems for operating land-based mariculture systems, such as the high costs of effluent treatment. This often tips the balance between profit and failure of sustainable mariculture production. The development of cost-effective biofilters for effluent treatment has the potential to solve both economic and environmental constraints. Periphyton represents a new class of photosynthetic-based biofilters known to be capable of removing many inorganic nutrients from aquaculture waste with minimal aeration costs. Periphyton is an aggregate of aquatic organisms that develop naturally while attached to a submerged substrate. The macroalgae *Ulva lactuca* is highly efficient for removal of ammonia (as TAN) in mariculture effluent but nitrate reduction may be limited even at relatively low levels of TAN. A recent study at the National Center for Mariculture (Eilat, Israel) aimed the potential of periphyton biofilter for mariculture effluent treatment. In this study periphyton revealed high uptake rates of TAN and DIN. Significant results were also generated by the addition of silica to the effluent for increasing diatoms prevalence, postponing appearance of macroalgae and increasing the lipid content in the produced biomass. Recently, a novel dual biofilter of Ulvaperiphyton was established in order to increase nitrogen removal. The dual *Ulvaperiphyton* biofilter removed up to 60% of the total nitrogen with specific removal efficiency of 92% of the TAN and of 55% of the TOXN as NO₃-N. Periphyton revealed no significant preference to either ammonia or nitrate by means of uptake rate and of removal efficiency. Highest NO₃ removal (39%) by the downstream periphyton was measured when TAN levels in effluent of the *Ulva* compartment were the lowest (below 0.16 mg L⁻¹). Removal efficiency of total nitrogen (TAN and TOXN) by the dual biofilter was 2-6 times greater than in a periphyton-only biofilters and between 1.5–2 times greater than in an *Ulva*-only biofilter. The results indicate that *Ulva* contributed predominantly to TAN removal while the periphyton provided supplemental nitrogen removal, predominantly of NO₃-N.

27. Macroalgae cultivation in Norway: Research on industrial development and environmental impact with relevance for guidelines

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An interdisciplinary knowledge platform for seaweed cultivation in Norway (MACROSEA) that is under development will be presented. Priorities are to increase the knowledge on biological performance and environmental requirements for optimized biomass production and chemical composition, and development of technological solutions and generic model simulation tools for farm systems and optimal growth. The talk will address synergies with work in progress to develop a vessel for industrial seaweed cultivation (SEAWEED VESSEL) and quantify positive and negative impacts on coastal ecosystem of industrial seaweed production (KELPPRO). Issues raised by the Norwegian Environmental Agency regarding seaweed cultivation will be presented.

28. Norwegian Integrated Multi-Trophic Aquaculture (IMTA) from pilotscale to a profitable industry

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The Norwegian government has set strategic goals for a fivefold increase in seafood production from aquaculture by 2050 (Olafsen et al., 2012). With marine-based bioeconomies on the rise globally, optimization of salmon aquaculture represents one example of ongoing efforts to improve production systems towards complete cycles of energy, water and resources. In this context, integrated multitrophic aquaculture (IMTA) systems have gained renewed attention, as they involve species from different trophic levels and „waste“ from one species becomes a resource for the other. In Norway, several experimental (pilot and small scale) IMTA systems with salmon and various species of kelp have been established and provide a starting point for investigating the potential benefits and disadvantages of co-culturing seaweed and fish (Stévant et al. 2016). However, to realistically evaluate the effects of integrated aquaculture on environmental and economic sustainability in seafood production, test sites must progress from pilot-scale to industrial scale. Upscaling will allow researchers and industry to investigate the technical, operational, economic and regulatory aspects of co-production.

The industry based research project “Tarelaks” is coordinated by the Norwegian seafood center and the research is led by Møreforsking AS. The aim is to estimate the effects of the integrated aquaculture methodology on fish production in standard operating conditions. The benefits and challenges of IMTA will be evaluated within three commercial salmon operations (Sulefisk AS/Hortimare AS, Engesund Fiskeoppdrett AS, Osland Havbruk AS) located in Western Norway. The framework of the programme is designed jointly for all three test sites (Furuholmen, Laberget, Bjønnspjotneset, respectively located in Solund, Masfjorden, and Høyanger municipalities). Salmon production of at least 1600t.yr⁻¹ is planned at each site, whereas the seaweed cultivation area will be gradually increased from 2ha to 10 ha after three to five years. The overall concept is designed for a 10yr time frame, with a detailed specific plan for the initial three-year period to reflect the fact that R&D licenses are granted for 3 years at a time, and that several research questions will need to be addressed over the long term. Research questions will be assessed in a spatial context (location specific), and at different time scales (diurnal, seasonal, annual). In the first few years, the research program will monitor: (i) fish production (biomass, environmental parameters, fish health and welfare), and (ii) seaweed production (e.g. species, seasonality, production technology). The first-year outcomes from this research program on the effects of IMTA of seaweed and salmon will be presented.

Keywords: industry, salmon, seaweed, fish health, environmental indicators

References:

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29 Macroalgal standardization in EU

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The European Union has realised that there is a need for standardisation of algae, due to the increased interest, but an area with no clear guidelines on how to e.g. describe dry biomass, analyse composition, and even identify species. There has been set a mandate to initiate the standardisation work within CEN 454 Algae. The preliminary report describing the priorities for the working groups was discussed at the kick off meeting in May 2017. But seaweed expertise is needed since the report clearly prioritised biofuels and microalgae. The CEN group is open for more expertise, so please join your national standardisation authorities if interested. Being involved in such work set the agenda, creates network and gives "license to trade" if you are from a company.

30. Breeding risks in aquaculture

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A major constraint on Seaweeds aquaculture is that growers must harvest wild individuals in order to obtain the material to be cultivated. From one species to another, populations from different localities can show different characteristics (morphology, chemical amounts...) as a response to the different environmental parameters. In that context, one main challenge for growers is to cultivate individuals that hold the best value for the traits of interest and that are well adapted to their local environment.

So, three of the main key points for breeding programs are: i) the compatibility between wild populations in terms of reproduction, ii) the environmental determinism for the traits of interest and iii) the genetic diversity of the cultivated population.

While these questions are well explored on higher plants, they are quite ignored in seaweeds.

The aim of this talk is to bring some theoretical and basic principles in order to supply the discussion about the Breeding risks in aquaculture.

31. Access and use of marine resources for scientific research, legislation and ethics

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Marine resources have the potential to sustainably deliver considerable wealth and business opportunities to local economies. Algae are already widely commercialized and many products are already on the market and are used by humans for wide range of applications such as food, renewable fuel, cosmetics, nutraceuticals, pharmaceuticals and aquaculture application. Billions of dollars are being invested by companies in the research and development of algae-based technologies which enhance marine bioprospecting activities and aquaculture activities. With the latest technological development made in seaweed aquaculture, one can question what are the potential impact of advanced aquaculture development on the environment. Based on UNCLOS existing and upcoming regulations as well as regional conventions and Nagoya protocol, this presentation will shed some light on the access, farming and use of marine resources, in high sea or territorial waters, providing robust ethical context for further bioprospecting or farming activities and will stimulate discussion on the risks of offshore innovative aquaculture development offshore

Participants of the Meeting

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